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CHAPTER 2

Experimental Models for the Analysis of IL-10 Function

Carl G. Feng, Julio Aliberti, Karl F. Hoffmann, Dragana Jankovic, Marika C. Kullberg, Alan Sher and Thomas A. Wynn

Abstract

This review focuses on the regulatory functions of IL-10 in the response to parasitic and bacterial infection revealed through knockout, cytokine/receptor blocking, and transgenic mouse studies. The various mechanisms that control the production and activity of IL-10 are also discussed. Studies performed over the past few years illustrate a complex and pleiotropic nature for IL-10 in host immunity. The fact that nearly every cell in the body can respond to IL-10 and multiple cells produce the cytokine likely explains this multifaceted activity. Studies conducted in experimental infectious and inflammatory diseases models have been particularly useful in defining the various regulatory activities of IL-10. Although these studies have identified many common themes for IL-10 in host immunity, they also nicely illustrate how IL-10 fine-tunes the response to individual pathogens and prevents inflammation.

Introduction

$CD4^+$ T helper (Th) cells can be divided into three major subsets, Type-1, Type-2 and Th3/T regulatory (Treg), based upon the specific cytokines produced and the functional activities exhibited by each cell type. Type-1 Th cells produce interferon-γ (IFN-γ) and lymphotoxin (LT), which promote macrophage activation and the generation of cell-mediated immunity. Type-2 Th cells produce a variety of cytokines including IL-4, IL-5, and IL-13, and provide help for the maturation of B cells to immunoglobulin-secreting cells, thereby activating humoral defense mechanisms. In contrast to Th1 and Th2 cells, however, T regulatory cells represent a unique and more heterogeneous population, which can express a variety of immune suppressive factors including CTLA-4, TGF-β, and/or IL-10.

Central to the concept of T helper subset generation is the tendency for an immune response to become polarized. Thus, a Type-1 or Type-2 cytokine-producing profile will often dominate quickly during an immune response by preferentially amplifying one Th subset while down regulating the opposing response. This polarized response appears to be critical for host defense against many pathogenic organisms. Resistance to intracellular pathogens often requires a predominantly Type-1 response, while Type-2 responses are typically needed to fight extracellular parasites. A primary goal of immunological research over the past decade has been to understand the various mechanisms that influence the polarization of the immune response following infection and to exploit those mechanisms in vaccine design. Whereas a polarized response is often required to control infections, there is also a need to balance the response. The various effector molecules, particularly those associated with the Th1 pathway, are nonspecific in their action and can be detrimental if produced for too long, in excess, or in the wrong location. The potentially harmful molecules include nitric oxide (NO), reactive oxygen intermediates (ROI), IL-1, IFN-γ, and TNF, and these factors often operate in a synergistic fashion.

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Therefore, it is important to produce a sufficiently potent type 1 response to provide efficient protection from infection, while at the same time producing a regulatory type 2 or immunosuppressive Treg cell response to prevent the protective response from causing damage to host tissues. Conversely, excessive Th2 response must also be dampened to prevent acute anaphylactic inflammation. The sections that follow illustrate how IL-10 regulates Th1 and Th2 response to infection.

IL-10 and Th1/Th2 Effector Choice

IL-10 was initially characterized as a Th2-specific cytokine that inhibits IFN-γ secretion by Th1 cells [1]. Because IL-10 can also be produced by activated antigen presenting cells (APC) (macrophages, dendritic cells (DC) and B lymphocytes [2-4]) it was regarded as a candidate factor that could positively influence the development of Th2 cells and negatively regulate differentiation of Th1 cells. However, experimental data have failed to support this simplistic view of IL-10's effect on Th1/T2 polarization. As anticipated, when primed with model antigens (Ag) or pathogens known to induce Th1-type responses, IL-$10^{-/-}$ animals display highly augmented immune responses frequently associated with detrimental Th1-mediated pathology. For example, IL-$10^{-/-}$ mice infected with *Toxoplasma gondii,* [5] *Plasmodium chaubudi,*[6] or certain strains of *Trypanozoma cruzi,* [7] have greatly elevated levels of IFN-γ, IL-12 and TNF-α and reduced parasitemia, but substantially increased risk of death from a toxic shock-like syndrome compared to WT (WT) controls. Unexpectedly, however, IL-$10^{-/-}$ mice also display enhanced Th2 responses when either challenged with allergens or exposed to Th2-type pathogens. [8-10] Together these findings show that IL-10 acts as a general negative regulator of CD4-dependent immune responses rather than a polarizing cytokine that influences Th1/Th2 commitment.

The inhibitory effect of IL-10 stems from its ability to down-regulate antigen-presenting functions of both macrophages and DC, the primary sources of Ag/MHC complexes during T cell priming. [11,12] The indirect influence of IL-10 on Th cells has been further supported by the analysis of IL-10R expression. IL-10R is expressed by most hematopoietic cells.[13] However, while its expression is down-regulated on activated $CD4^+$ T lymphocytes, [14] activation of monocytes is associated with an increase in IL-10R levels, [15] providing the molecular basis for the IL-10 responsiveness of the latter but not the former cell population.

In the context of Th effector choice, an important aspect of IL-10 effects on APC is its ability to inhibit not only the expression of MHC class II and costimulatory molecules but also the secretion of cytokines and chemokines.[12,16] Although the latter effect of IL-10 is not selective and affects most of the soluble mediators produced by activated macrophages and DC, its primary consequence is down-regulation of the Th1 development, because many of the monokines (e.g., IL-12, IL-18, IL-23 and IL-27) are IFN-γ inducible cytokines required for optimal Th1 differentiation.[17] For the same reason, IL-10-treated macrophages or DC appear to be promoting Th2 development. [18] In contrast to this differential effect on Th1/Th2 differentiation, the accumulation of mature Th1 and Th2 effectors at the site of inflammation can be equally affected by IL-10 since it down-regulates the production of both CC and CXC chemokines. [19,20] In addition to inhibiting the production of cytokines and chemokines, IL-10 also enhances the expression of their natural antagonists by increasing the expression of either decoy (e.g., IL-1RA and chemokine receptors) [21,22] or soluble (e.g., p55 and p75 TNFR) receptors [23,24] that in turn potentiate IL-10's down-modulatory effects on APC functions.

Different IL-10-producing DC populations (e.g., from Peyer's patches [25] and liver [26]) have been associated with the development of Th2 responses. Recently, these observations have been extended by the findings that IL-10 is required for optimal development of Th2 cells by the $CD8^-CD11c^+$ subset of splenic DC. [27] However, since IL-10 may selectively induce apoptosis of $CD8\alpha^+$ $CD11c^+$ cells, [27] this Th2 priming by IL-10 appears to be a result of a loss of IL-12-producing DC and a subsequent lack of Th1 differentiation. In addition, while the particular DC subsets were not analyzed, naïve and *Trichinella spiralis*-infected IL-10 knockout (KO) mice display higher number of $CD11c^+$ DC in mesenteric lymph nodes when compared

to WT animals.[28] Autocrine IL-10 has been shown to prevent spontaneous maturation of human DC in vitro and to limit LPS and CD40-induced maturation.[29]

While initially specifically associated with Th2 cells, the expression of IL-10 is now found in other Th subsets as well. When cultured in the presence of IL-10, murine bone marrow-derived DC promote development of IL-10$^+$ CD4$^+$ Treg lymphocytes. [30] Moreover, similar to human Th1 cells, [31] murine Th1 lymphocytes may also coexpress IL-10. For example, "classical" murine Th1 immune responses following infection with different intracellular pathogens (e.g., *Brucella abortus*, *Borrelia burgdorferi*, *Leishmania major*, *T. gondii*) include not only IFN-γ^+ CD4$^+$ cells but also "nonclassical" Th1 lymphocytes that concomitantly produce IFN-γ and IL-10 [32-35]

Thus, although the effect of IL-10 on Th1/Th2 effector choice is indirect and very complex (Fig. 1), IL-10 and IL-10R still represent attractive therapeutic targets for the manipulation of APC function aimed at both promoting or/and suppressing development of different types of CD4-dependent immune responses.[36,37]

IL-10 in Schistosome Infection

Like most host/helminth relationships, schistosome worms and their definitive hosts have coevolved survival strategies that maximize the transmission of parasite gametes (enclosed in the developing egg) and minimize the development of pathology in the host. While these strategies work well for the vast majority of the 200 million people currently infected with this pathogen, a small proportion of those affected will go on to develop life-threatening or severely debilitating illnesses. [38] Although there are many confounding factors that influence the schistosome/host equilibrium and clinical outcome, the induction of IL-10 during infection is a vital and indispensable process that limits host pathology and facilitates long-term survival of the parasite and host.

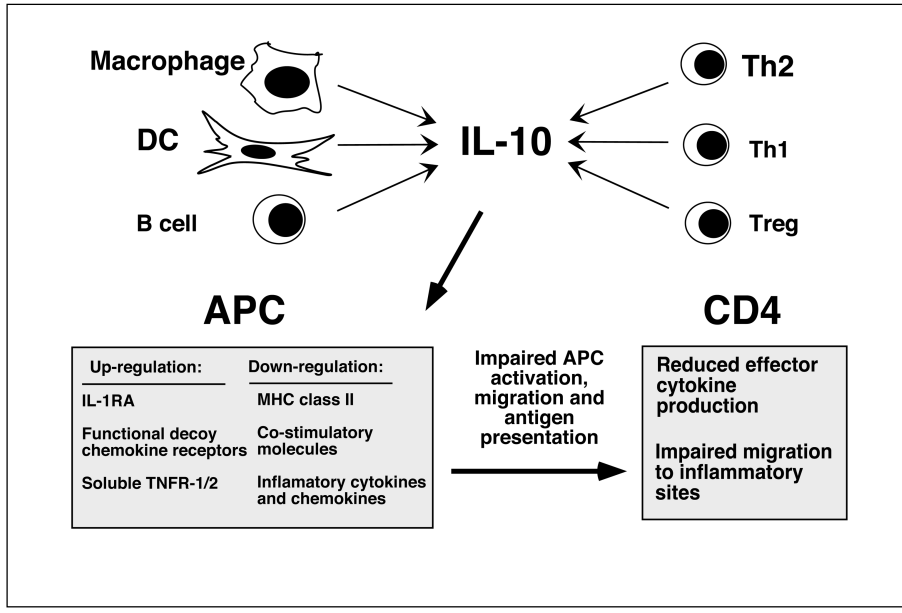

Figure 1.

Schistosome cercariae release proteases as they penetrate the skin of their definitive host – a process that leads to damage of surrounding tissues and the generation of robust innate immune defense mechanisms. However, greater than 90% of infective stage cercariae survive this process and ultimately reach the lungs. [39] Prostaglandins induced and released by the cercariae [40,41] are believed to be indirectly responsible for increased schistosome survival during this critical period of infection via their effect on the host's immune system. Specifically, one prostaglandin, prostaglandin E_2 (PGE_2) , up-regulates the production of keratinocyte-derived IL-10, which in turn limits the induction of anti-parasite inflammatory reactions in the skin of experimentally infected animals. [40] The production of IL-10 in skin seems to occur regardless of the parasite species used during the infection [42] and is also observed in lymph nodes draining the skin. [43] Moreover, studies in vaccinated IL-$10^{-/-}$ mice demonstrated that IL-10 dampens nearly all known anti-parasite effector mechanisms that operate during polarized Th1 and Th2 responses. [44] Finally, a recent study of *S. haematobium* infected children identified IL-10 as a major risk factor for reinfection after chemotherapy. [45] Together, the results of these studies suggest that schistosome parasites have evolved an IL-10-dependent mechanism that down-regulates the host's immune response early during infection, which maximizes their survival. However, it is also clear that IL-10 is critical to the survival of the infected host, by limiting egg-induced liver damage as infection becomes chronic.

Deposition of schistosome eggs into the intestines and liver of infected hosts induces a vigorous Th2 mediated, circumoval granulomatous response that, if not properly controlled, can lead to severe immuno-pathology. [38] Glycoconjugates and lipids derived from schistosome eggs [46-48] drive IL-10 production from B cells, [49] other APCs [48]), and Treg cells (Ref [47] and Hesse M, Piccirillo CA, Belkaid Y, Prufer J, Mentink-Kane M, Leusink M, Cheever AW, Shevach EM and Wynn TA. The pathogenesis of schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. Submitted) possibly through a p38 protein kinase dependent signaling cascade. [50] IL-10 levels remain high even weeks after the egg-induced process is initiated and associate with global T cell hyporesponsiveness, [51-54] counter regulation of inflammatory Th1 cell populations, [51,55] decreased proliferative capacity of host cells, [53,56] and control of circumoval granulomatous responses. [55,57,58] Together, these IL-10 dependent activities create an environment that prevents the formation of an over exuberant and potentially dangerous anti-egg inflammatory response.

Further insight into the regulatory role of IL-10 during schistosome infection has recently been uncovered through studies involving experimentally infected IL-10 deficient mice and analysis of data obtained from human immuno-epidemiological field investigations. As IL-10 is associated with the control of the granulomatous response and host cell proliferation during schistosome infection, it was suggested that this cytokine might be important for down-modulation of host circumoval immune responses during chronic infection. Nevertheless, a longitudinal study using schistosome-infected IL-10 deficient animals demonstrated that the magnitude of the granulomatous response decreases substantially between wk 8 and 16 of infection, [10] suggesting that IL-10 plays only a minor role in the process of immune down-modulation. Nevertheless, further examination of the immune responses in these animals as well as double gene deficient mice (IL-10/IL-12- and IL-10/IL-4- KO) demonstrated that IL-10 critically controls Th1 and Th2 cytokine and antibody responses as well as immunopathology, especially during the acute phase of disease. [10,59] Deficiencies in IL-10 are also associated with increased pathology in infected CBA/J mice, [60] IL-4 deficient mice, [61] mice made tolerant to egg antigens, [62] $CD4^+$ T cell-depleted mice, [63] mice coinfected with *S. mansoni* and *T. gondii* , [64] and in mice immunized with egg antigens and complete Freund's adjuvant. [65] Furthermore, IL-10 also plays an important role in the development of egg-induced hepatic fibrosis by regulating IL-13Rα2 expression (decoy receptor for the collagen inducing cytokine IL-13). [66] Together these studies indicate that IL-10 production during experimental schistosomiasis is important for several infection-related pathologies. [67]

Can IL-10 contribute to the control of severe morbidity in human populations? To begin to answer this question, one recent study has elegantly confirmed the role of IL-10 in urinary tract morbidity during *S. haematobium* infection of children and adolescents in Kenya. [68] Here, the authors demonstrated that a low ratio of IL-10/TNF-α positively correlated with severe bladder wall pathology in the age- and infection intensity- matched case population. In another study performed on the shores of Lake Albert in Uganda, low levels of IL-10 were positively associated with increased fibrosis in children infected with *S. Mansoni.* [69] Additional studies of this type will contribute to our understanding of the role of IL-10 in human schistosomiasis and other helminth infections. [28,70,71] Interestingly, a beneficial side effect of prolonged helminth-induced IL-10 production in chronically infected individuals is the ability of this cytokine to suppress atopy. [72] Given the many critical functions exhibited by IL-10 in this disease, It is clear that interest on IL-10 and other IL-10 related family members [73] will continue to grow in the coming years.

IL-10 in Intracellular Protozoan Infection

Due to their capacity to induce vigorous pro-inflammatory cytokine production, protozoan pathogens such as *L. major,* [74] *T. cruzi* [75] and *T. gondii* [76,77] rapidly stimulate IL-10 responses. This response quickly establishes an important equilibrium that limits damage to the host but at the same time prevents complete clearance of the organism so that transmission to new hosts can occur. Surprisingly, however, little is know about the stimuli that trigger IL-10 production in these infections or which cell types produce the cytokine. It is now widely believed that $CD4^+CD25^+$ Treg cells represent a major source of the cytokine during infection with *L. major.* [34,78] While the mechanisms that drive APC to produce IL-10 during *L. major* infection are not completely clear, it was found that IgG bound to amastigote forms by means of Fc receptor ligation can stimulate IL-10 production .[79] In the case of *T. cruzi*, some parasite membrane-derived glycoinositolphopholipids possess anti-inflammatory activity on macrophages and DC in vitro, but this effect does not appear to be due to induction of IL-10. [80] Interestingly, while DC fail to secrete IL-10 in response to *T. gondii* stimulation, T cells, macrophages and glial cells produce significant levels of the cytokine during in vivo infections.

IL-10 was originally thought to regulate resistance to protozoan infection mostly through effector cell deactivation such as by inhibiting NO expression by macrophages [81,82] or by immune deviation of T cell responses towards a type 2 cytokine profile. [83] However, when $IL\text{-}10^{-/-}$ mice became available, this paradigm had to be modified to accommodate a wider range of effects of this cytokine during infection. *T. gondii* and *T. cruzi* infection in $IL\text{-}10^{-/-}$ mice resulted in an enhanced Type 1 response and lower parasite burdens as expected, but also revealed a much more unpredicted outcome of excessive inflammation, which was associated with tissue destruction and a lethal shock-like syndrome characterized by over-production of IL-12, IFN-γ and TNF. [5,7,84,85] An additional mechanism by which IL-10 can control inflammation is through direct inhibition of chemokine expression induced by the parasite. [86]

L. major infection in $IL\text{-}10^{-/-}$ mice results in complete clearance of the parasites from skin lesions, suggesting a role for this cytokine in the induction of parasite persistence. [34,79] Belkaid and colleagues reported that $CD4^+CD25^+$ Treg cells are the major cell population secreting IL-10 and, therefore, regulating chronic persistence of leishmania parasites. [78] They hypothesized that direct inhibition of microbicidal activity by the IL-10 produced by this T cell population led to the persistence of the parasite. Nevertheless, a role for TGF-β Another immunomodulatory cytokine produced by Treg cells, has not been formally excluded. TGF-β has been shown to be associated with macrophage deactivation, inhibition of microbicidal function and proinflammatory mediators release in several models of protozoan infection. [87-90]

Metabolites of the arachidonic acid also constitute another group of anti-inflammatory mediators that can regulate immunity against protozoa parasite infections. PGE_2 production was reported in mice infected with L. major and T. cruzi. [91-94] A more direct correlation between production of PGE_2 and susceptibility to infection was observed after in vivo inhibition

of PGE_2 synthesis by treatment with cyclooxygenase inhibitors. [91,92] Nevertheless, the most common cyclooxygenase inhibitors, such as Indomethacin were also shown to inhibit lipoxygenases, [95] a second class of enzymes that trigger the release of other immunomodulatory mediators. Leukotriene B_4 (LTB_4) is one of the products of the lipoxygenase metabolism of the arachidonic acid, its production had been reported during infection with *L. major* [93] that appears to have an enhancing effect over cytokine production, independently of their anti- or pro-inflammatory profile. IL-10-independent regulation of IL-12 and IFN-γ production was also reported after stimulation of mice with an extract of tachyzoites of *T. gondii,* [96] a phenomenon called "dendritic cell paralysis". It was found later that in vivo stimulation with this parasite extract induced the release of a 5-lipoxygenase-derived eicosanoid, lipoxin A_4 (LXA_4) and that 5-lipoxygenase deficient mice can not secrete LXA_4 or undergo dendritic cell paralysis. [97] The in vivo relevance of LXA_4-mediated control of IL-12 production was studied during infection of 5-lipoxygenase deficient mice with *T. gondii.* [98] These animals succumbed to infection around 30 days post-inoculation with a lower parasite burden, higher serum IL-12 levels and intense inflammation in the brain with elevated IL-12 production in situ. [98] However, when analyzed in parallel in an in vitro study, IL-10 but not LXA_4, was effective in blocking macrophage microbicidal function, suggesting that these mediators have related but not redundant effector pathways.

IL-10 in Mycobacterial Infection

Mycobacteria are slow-growing, facultative intracellular bacilli that primarily reside in phagocytes. The immune response to mycobacteria has been analyzed extensively in mouse models of *Mycobacterium tuberculosis, M. bovis* Bacillus Calmette-Guérin (BCG) and *M. avium* infections. Activation of infected macrophages and control of mycobacterial replication is critically dependent on IFN-γ produced by T lymphocytes. [99] Some bacilli, however, resist killing and survive within macrophages in the face of strong T cell responses. Although it is unclear how this latent infection is maintained, mechanisms that alter host immune responses, such as the induction of down-regulatory cytokines like IL-10 and TGF-β are thought to contribute to the persistence of mycobacterial infection. Production of IL-10 is of special interest as a possible evasion strategy because of its suppressive effects on many known immune functions required for inhibiting mycobacterial growth, including synthesis of pro-inflammatory cytokines/mediators, expression of MHC class II and costimulatory molecules. [15]

IL-10 is strongly induced at the sites of mycobacterial infection. [100-102] APC, such as macrophages and DC, [103-105] as well as T lymphocytes [106,107] are capable of producing IL-10 in response to mycobacterial infection. Interestingly, although originally described as a Th2 cytokine, IL-10 also appears to be produced in large quantities by Th1 IFN-γ-producing $CD4^+$ lymphocytes during mycobacterial infection. [108,109]

IL-10 inhibits cellular responses induced by mycobacterial infection at multiple levels. After activation with IFN-γ__murine macrophages release pro-inflammatory cytokines and NO to control the intracellular growth of *M. tuberculosis* and *M. bovis.* [110-113] This IFN-γ-mediated bactericidal effect, however, is inhibited in the presence of IL-10. [114] Moreover, IL-10 prevents TNF-dependent apoptosis of *M. tuberculosis*-infected macrophages by inhibiting TNF production [115] or by inducing the release of TNF receptor 2 that could form nonactive TNF-TNFR2 complexes. [116,117] The induction of macrophage apoptosis may restrict mycobacterial spreading [118] as well as facilitate antigen presentation to T cells [119] thereby contributing to host control of the infection. Although IL-10 does not exhibit a direct suppressive effect on Th1 cells (see previous section), the cytokine may influence the T cell response to mycobacterial infection by modulating APC functions. Mycobacterium-induced IL-10 inhibits IL-12 production by DC in vitro and in vivo. [104,105] In addition, BCG-infected, IL-10-deficient DC have been shown to migrate more efficiently to draining lymph nodes compared to cells from WT mice, suggesting that autocrine IL-10 regulates DC migration in response to BCG infection in vivo. [104]

As noted above, since IL-10 has a major down-regulatory effect on cell-mediated immunity, it has been hypothesized that the production of this cytokine promotes the long-term survival of mycobacteria in infected hosts .[120-122] Initial studies, [123,124] which used neutralizing antibody to block IL-10 function in vivo, in general supported this concept. However, more recent studies employing IL-$10^{-/-}$ mice have yielded conflicting results (Table 1). For example, IL-$10^{-/-}$ mice show increased resistance to *M. avium* [125] and in some [125-127] but not all [102,128,129] studies display transiently enhanced control of *M. tuberculosis* and BCG infection. The discrepancy between these studies possibly results from variation in the virulence of the mycobacteria, the time-points analysed and most importantly, the route of infection.

Although IL-$10^{-/-}$ mice display only minimally enhanced resistance to mycobacterial infection, such observation does not rule out a role for IL-10 as one of several redundant mechanisms regulating host resistance to these microorganisms. It has been demonstrated that the over-expression of IL-10 in transgenic mice results in significantly impaired host resistance to *M. Tuberculosis,* [101] BCG [130,131] and *M. avium* [107] infection. Because the expression of transgenic IL-10 can be controlled by cell lineage-specific promoters, the relative effect of T cell- vs APC-derived IL-10 on the host immune response to mycobacterial infection was investigated. Over-production of IL-10 by T cells ,[101,130] macrophages [131] or MHC class II expressing cells [107] lead to dramatically elevated bacterial burdens and impaired macrophage functions. IFN-γ responses, however, were not markedly decreased in these infected transgenic animals, suggesting normal development of Th1 effector cells. Together, these observations are consistent with the in vitro findings that IL-10 can over-ride the macrophage activation effects of IFN-γ_ [114]

In conclusion, both in vivo and in vitro studies demonstrated that excessive IL-10 production can promote intracellular pathogen growth in macrophages and argue that IL-10-mediated immune down-regulation may contribute to the maintenance of latency in chronic mycobacterial infection, possibly as one of several redundant mechanisms.

Table 1. Effects of manipulation of IL-10 level on host resistance to mycobacterial infection

Mycobacterium Spp.	**Methods**	**Bacterial Burdens[a]**	**References**
M. avium	Anti-IL-10[b]	Reduced	Ref. [123, 124]
	IL-10 KO[c]	Reduced	Ref. [125]
	IL-10 Tg[d]	Increased	Ref. [107]
BCG	IL-10 KO	Unchanged	Ref. [128]
	IL-10 KO	Transiently reduced	Ref. [126, 127]
	IL-10 Tg	Increased	Ref. [130, 131]
M. tuberculosis	IL-10 KO	Unchanged	Ref. [102, 129]
	IL-10 KO	Transiently reduced	Ref. [125]
	IL-10 Tg	Increased	Ref. [101]

a. Compared to those in WT mice.

b. Treated with antibody specific for IL-10

c. IL-10 deficient mice

d. IL-10 transgenic mice

The Role of IL-10 in the Regulation of Inflammatory Bowel Disease

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is the major chronic inflammatory disease of the intestinal tract. Although the etiology of IBD is unknown, the intestinal flora is believed to play an important role in its pathogenesis. This is perhaps best illustrated in experimental models of the disease – e.g., the IL-10$^{-/-}$ mouse model – in which various immunodeficient animals develop intestinal inflammation when housed in conventional animal facilities, but not when reared under specific pathogen-free or germ-free conditions. (reviewed in 132)

The immune mechanisms that regulate intestinal inflammation have been extensively studied over the years, and data from the severe combined immunodeficiency (SCID) transfer model have been particularly useful in defining both pathogenic and disease-protective CD4$^+$ T cell responses in IBD. In the SCID transfer model, colitis is induced in T cell-deficient SCID or Rag$^{-/-}$ recipients by transfer of naïve CD4$^+$ CD45RB^{hi} T cells. [133,134] Cotransfer of the CD45RB^{low} memory T cell subset prevents the development of inflammation, defining a population of CD4$^+$ Treg cells with disease-suppressive function. [134-136] Subsequent studies have demonstrated that IL-10 plays a disease-protective role in this model as 1) systemic administration of recombinant IL-10 prevents development of CD45RB^{hi}-induced colitis, 2) anti-IL-10R treatment reverses the disease suppression mediated by the CD45RB^{low} cells, and 3) CD45RB^{low} cells from IL-10$^{-/-}$ animals fail to protect from disease. [137,138] Furthermore, CD45RB^{hi} cells isolated from IL-10 transgenic mice do not induce colitis in SCID recipients and these transgenic IL-10-secreting CD45RB^{hi} cells are even able to protect from colitis induced by CD45RB^{hi} cells from WT mice. [139]

IL-10 clearly controls intestinal inflammation also in other models of colitis. For example, IL-10 therapy has been shown beneficial in preventing and/or partially reversing disease in the IL-10$^{-/-}$ and trinitrobenzene sulphonic acid (TNBS) colitis models. [140,141] Moreover, Treg cell suppression of T-cell dependent as well as T-cell independent *Helicobacter hepaticus*-triggered intestinal inflammation in Rag$^{-/-}$ mice is reversed by anti-IL-10R treatment. [142,143] Interestingly, administration of anti-IL-10R mAb to normal BALB/c mice leads to the induction of colitis, [144] arguing that IL-10 is required also in intact immunocompetent animals to maintain intestinal homeostasis. Studies from the *H. hepaticus* colitis model have further demonstrated that whereas infected IL-10$^{-/-}$ animals develop a pathogenic Th1 type response, infected WT mice that are disease free mount an IL-10-dominated immune response against the bacterium. [145] These studies support the hypothesis that in immunocompetent hosts, intestinal flora induces IL-10-secreting CD4$^+$ T cells that prevent pathologic immune responses towards intestinal antigens. The cellular source of the disease-protective IL-10 in most of the colitis models are indeed believed to be CD4$^+$ Treg cells, [138,142,143,145] although B cell-derived IL-10 has been reported to suppress intestinal inflammation in TCRα-deficient mice. [146]

There are likely multiple mechanisms by which IL-10 exerts its disease-suppressive effect in IBD. Treg cells, through their production of IL-10, are known to control the expansion of colitogentic CD4$^+$ T cells. [138,142,143,147,148] Moreover, in addition to its down-regulatory effects on APC populations, [11,12,149] IL-10 has been shown to promote the development of IL-10-secreting CD4$^+$ Treg cells in vitro [150] and to enhance the differentiation of DC that prime such Treg cells. [30] Evidence that IL-10 may prevent intestinal inflammation by acting on the innate arm of the immune response comes from a report describing the development of enterocolitis in mice whose macrophages and neutrophils are rendered IL-10 unresponsive by specific disruption of the Stat3 gene. [151] Likewise, as mentioned above, Treg cells are able to in an IL-10-dependent fashion suppress the colitis that develops in *H. hepaticus*-infected Rag$^{-/-}$ mice on the 129SvEv background, suggesting that cells of nonT lymphocyte compartments are the targets of IL-10 activity. [143] Besides IL-10, TGF-β plays an important role in protection against colitis [152-154]. The relation between IL-10 and TGF-β in disease suppression is not yet clear, however studies in the TNBS colitis model suggest that IL-10 acts by down-regulating

the Th1 response, thereby facilitating TGF-β secretion in the host [155]. IL-10 may also enhance TGF-β receptor type II expression and restore TGF-β responsiveness of activated T cells [156].

Similar to the findings in experimental models, the gut flora has been implicated in the development of IBD also in humans [157-159]. Moreover, while normal individuals display peripheral tolerance against resident autologous flora mediated by $CD4^+$ T cells secreting IL-10 and TGF-β_ [160] this state is broken in active IBD. [161] In contrast to rodent models, however, systemic treatment of IBD patients with recombinant human IL-10 has thus far not been very effective, and other approaches are therefore being developed for use in humans. [162] Encouraging results have been obtained from experimental models using IL-10-secreting *Lactococcus lactis* to treat $IL\text{-}10^{-/-}$ mice as well as mice exposed to dextran sodium sulfate, [163] and a phase I clinical trial using IL-10-secreting bacteria in patients with Crohn's disease is currently underway. [162] Taken together, IL-10 clearly has suppressive effects on inflammatory responses in the intestine and with improved methods for delivery this cytokine may prove beneficial as a treatment for humans with IBD.

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